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Lamin A deregulation in human mesenchymal stem cells promotes an impairment in their chondrogenic potential and imbalance in their response to oxidative stress

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Abstract In the present study, we examined the effect of the over-expression of LMNA, or its mutant form progerin (PG), on the mesoderm differentiation potential of mesenchymal stem cells (MSCs) from human umbilical cord (UC) stroma using a recently described differentiation model employing spheroid formation.

Accumulation of lamin A (LMNA) was previously associated with the osteoarthritis (OA) chondrocyte phenotype. Mutations of this protein are linked to laminopathies and specifically to Hutchinson–Gilford Progeria Syndrome (HGPS), an accelerated aging disease. Some authors have proposed that a deregulation of LMNA affects the differentiation potential of stem cells.

The chondrogenic potential is defective in PG-MSCs, although both PG and LMNA transduced MSCs, have an increase in hypertrophy markers during chondrogenic differentiation. Furthermore, both PG and LMNA-MSCs showed a decrease in manganese superoxide dismutase (MnSODM), an increase of mitochondrial MnSODM-dependent reactive oxygen species (ROS) and alterations in their migration capacity. Finally, defects in chondrogenesis are partially reversed by periodic incubation with ROS-scavenger agent that mimics MnSODM effect.

Our results indicate that over-expression of LMNA or PG by lentiviral gene delivery leads to defects in chondrogenic differentiation potential partially due to an imbalance in oxidative stress.

Abbreviations: MSCs, mesenchymal stem cells; LMNA, lamin A; PG, progerin; UC, umbilical cord; HGPS, Hutchinson–Gilford Progeria Syndrome; OA, osteoarthritis; ROS, reactive oxygen species; MnSODM, manganese superoxide dismutase; ECM, extracellular matrix; DMEM, Dulbecco's Modified Eagles Medium; GFP, green fluorescent protein; RT-PCR, real-time polymerase chain reaction; LPL, lipoprotein lipase; FABP, fatty acid binding protein; ADIPOQ, adiponectin; ALP, alkaline phosphatase; OC, osteocalcin; AGG, aggrecan; RPLP, Ribosomal Protein Large P1; SDF-1, stromal cell-derived factor 1; IL-8, interleukin 8.

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Introduction

MSCs are multipotent cells having the capacity to differentiate into various cell lineages, some of which generate bone, cartilage and adipose tissue (Arufe et al., 2011). Cells expressing MSC markers have been found in many tissues including the synovial membrane and cartilage tissues (Hermida-Gómez et al., 2011). In addition, osteoarthritic synovial membrane and cartilage contain more cells expressing MSC markers than do synovial membranes from healthy joints (Nagase et al., 2008). MSCs are likely to be agents of connective tissue homeostasis and repair.

Lamin A and lamin C (A-type lamins, both encoded by the LMNA gene) are major components of the mammalian nuclear lamina, a complex pertinacious structure that acts as a scaffold for protein complexes that regulate nuclear structure and function (Gruenbaum et al., 2003; Prokocimer et al., 2009). Mutations in the LMNA gene play a key role in the pathogenesis of a group of diseases called laminopathies, affecting mesoderm tissues (Worman et al., 2010). One of the most studied laminopathies is Hutchinson–Gilford Progeria Syndrome (HGPS) which is due, in most of the cases, to nuclear accumulation of a permanently farnesylated, mutant form of prelamin A called progerin (PG) (Eriksson et al., 2003). HGPS is a very rare and fatal genetic disorder characterized by cellular senescence and an early onset of pathologies typical of advanced age such as atherosclerosis, myocardial infarction, stroke or OA (Hennekam, 2006). Remarkably, PG is also present in normal cells and appears to progressively accumulate during aging of non-HGPS cells (Wang et al., 2008). Besides producing structural defects in the nuclear lamina, it has also been suggested that PG may interfere with the proposed gene regulatory function of lamins, specifically the regulation of the p16/Rb pathway necessary to maintain balance between differentiation and proliferation of multipotent mesenchymal stromal or stem cells (MSCs) in tissue regeneration (Espada et al., 2008; Scaffidi and Misteli, 2008; Hernandez et al., 2010).

Recently, increased accumulation of lamin A in osteoarthritis (OA) chondrocytes and cartilage (Ruiz-Romero et al., 2008; Attur et al., 2012) has been reported. OA is one of the most common skeletal disorders clinically manifested by joint pain, swelling and progressive loss of function. It is characterized by cartilage degradation, hypertrophy of the subchondral bone and osteophyte formation at the joint margins (Lamas et al., 2010). Although the underlying molecular mechanisms involved in the disease remain unknown, the etiology of OA has been considered an articular cartilage disorder induced by mechanical stress, articular injuries, involvement of inflammatory mediators and aging. Evidence suggests that cellular senescence of the chondrocyte is inherent to the OA process (Loeser, 2011). Finally, progression of the disease has been shown to be therapeutically modulated by MSCs (Mafi et al., 2011; van Buul et al., 2012).

The aim of this study was to demonstrate whether lamin A deregulation in MSCs modulates the chondrogenesis process. We demonstrated that over-expression of lamin A and PG reduces the chondrogenic capacity of MSC. We also provided evidence that linked those defects to alterations in oxidative stress response through using N-acetylcysteine which protects against hypoxia produced by production of oxygen reactive species (Cillero-Pastor et al., 2008).

Material and methods

Tissue collection

Human UCs were obtained from cesarean sections performed on healthy women at the Maternity Facility of Complejo Hospitalario Universitario A Coruña (CHUAC). All tissues were obtained with fully informed consent and ethical approval by the supervisor of the Ethical Committee (CEIC) of Galicia. The UC donors were between 26 and 35 years-old.

Isolation and culture of MSCs

MSCs were isolated from UC stromal tissue using a previously described protocol (Arufe, de la Fuente et al., 2011). Briefly, the tissue was washed with phosphate-buffered saline (PBS) and cut in small pieces, termed "explants;" which were incubated for three five-minute periods in an enzymatic cocktail containing 1.2 U/mL dispase and 112 U/mL collagenase type I (Sigma-Aldrich, St. Louis, Missouri, USA). The explants were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin (Sigma-Aldrich) until adhered to the plastic plate. After three days, the explants were removed, leaving the attached UC MSCs, which continued monolayer culture in the same medium. When the cells were 90% confluent, they were removed from the plate using 2% trypsin (Sigma-Aldrich) in PBS and induced to differentiate toward mesoderm cell lines. The cells were characterized by flow cytometry using a FACSAria flow cytometer (BD Bioscience, Madrid, SP) to check the following surface markers: PE-conjugated anti-human CD73 (1:20 from BD Pharmingen); PE-Cy5.5-conjugated mouse anti-human CD90 (1:20 from BD Pharmingen); FITC mouse anti-human CD105: (1:100 from Serotec, Bavaria, Germany); FITC mouse anti-rat CD45 (1:20 BD Pharmingen) and CD34 (1:20 from DakoCytomation, Barcelona, SP). FACS data were generated by DIVA software (BD Bioscience). Negative control staining was performed using FITC-conjugated mouse IgG1k isotype, PE-conjugated mouse IgG1k isotype and PE-Cy5.5-conjugated mouse IgG1k isotype (all from BD Pharmingen). Cells were fixed in 4% paraformaldehyde in PBS. After fixation, the cells were washed twice with PBS, permeabilized with PBS containing 0.1% Triton X-100 (Sigma-Aldrich) for 10 min, then pre-blocked with 3% bovine serum albumin (BSA) and incubated with the different antibodies tested for 30 min at room temperature. The stained cells were then washed twice with PBS and 1×10^5 cells were analyzed.

Cloning procedure

Full length human PG, LMNA and green fluorescent protein (GFP) cDNAs were amplified by polymerase chain reaction (PCR) from pBABE-puroGFP-progerin and pBABE-puroGFP-lamin A plasmids (Addgene, donated by Tom Misteli), using the oligonucleotides EcoRI-LMNA-forward: CCGGAATTCATGGAGA CCCCGTCCCAGCGG, BamHI-LMNA-reverse: CGCGGATCCTTACA TGATGCTGCAGTTCTG, EcoRI-GFP-forward: CCGGAATTCATGG TGAGCAAGGGCGAG, BamHI-GFP-reverse: CGCGGATCCTTACTT GTACACCTCGTC. GFP, LMNA and PG were cloned into pLVX-puro (Clontech Laboratories Inc., Mountain View, CA) in